ECBC-TR-1011

DISINFECTION OF AIRBORNE ORGANISMS
BY ULTRAVIOLET-C RADIATION AND SUNLIGHT

Jana Kesavan
Jose-Luis Sagripanti

RESEARCH AND TECHNOLOGY DIRECTORATE

July 2012

U.S. ARMY RDECOM

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
XX-07-2012	Final	Sep 2011 - Jun 2012

4. TITLE AND SUBTITLE

Disinfection of Airborne Organisms by Ultraviolet-C Radiation and Sunlight

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Kesavan, Jana; and Sagripanti, Jose-Luis

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Director, ECBC, ATTN: RDCB-DRI-A\\RDCB-DR, APG, MD 21010-5424

8. PERFORMING ORGANIZATION REPORT NUMBER

ECBC-TR-1011

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT-LIMIT 200 WORDS

This report provides background information on UV radiation, selected organisms of potential interest to aerosol researchers, and an overview of the effects of UV light on aerosolized organisms. Aerosol generation methodology and equipment, as well as test methodology used at U.S. Army Edgewood Chemical Biological Center (ECBC), are provided as examples for researchers to compare to their own methodology or as guidance for those newer to this field.

15. SUBJECT TERMS

UV radiation   Sunlight   Bio-organisms   Decontamination   Kill curves

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Renu B. Rastogi
U	U	U	UU	34	**19b. TELEPHONE NUMBER** *(include area code)* (410) 436-7545

Blank

PREFACE

The work described in this report was started in September 2011 and completed in June 2012.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release.

Blank

CONTENTS

FIGURES

TABLE

DISINFECTION OF AIRBORNE ORGANISMS
BY ULTRAVIOLET-C RADIATION AND SUNLIGHT

1.   INTRODUCTION

Bioaerosols are a serious concern to human health because of their potential role in transmission of infectious diseases during natural epidemics or after intentional release of biological agents through acts of terrorism or warfare. Extensive research has been conducted toward understanding the disinfection of organisms deposited on surfaces, suspended in water, and contaminating food, all of which have been discussed elsewhere (Block, 2001). In contrast, the disinfection of microorganisms in aerosols has received much less attention, mainly because of the difficulties inherent in conducting the experiments. Airborne organisms are difficult to reach with the liquid disinfectants that are commonly used to treat medical devices, foods, and drinking water. Therefore, the primary means for organism inactivation in aerosols is ultraviolet (UV) radiation.

Radiation from the sunlight is used as a disinfectant to kill organisms. Radiation of <290 nm wavelength is absorbed by atmospheric ozone before sunlight reaches the earth's surface; therefore, the longer UV wavelengths in sunlight provide the main germicidal agent in the environment (Jagger, 1985; Giese, 1976; Lytle and Sagripanti, 2005). Germicidal UV lights are relatively easy to use indoors; hence, light with 254 nm wavelength is frequently used to disinfect the air and surfaces in healthcare and biomedical industrial settings.

Organisms appear to be more susceptible to UV radiation when they are suspended in air (King et al., 2011). For this reason, conclusions derived from studies of organisms on liquids or surfaces are generally not transferable to aerosolized organisms. This increased susceptibility is likely a result of comparatively easier mixing in air combined with lower rates of UV absorption by air. In addition, the aerosolization process itself may weaken or damage the organisms, thereby rendering them more susceptible to UV irradiation. The effects of dehydration and oxygenation may also contribute to the increased vulnerability of airborne microbes.

This report provides background and overview information on UV radiation, selected organisms of potential interest to aerosol scientists and engineers, and the effects of UV light on aerosolized organisms. The aerosol generation methodology and equipment, as well as the test methodology used at the U.S. Army Edgewood Chemical Biological Center (ECBC), are provided as examples for researchers to compare to their own experimental conditions or as guidance to those newer to this field.

2.   UV RADIATION

Electromagnetic radiation is a fluctuation of electric and magnetic fields in space and is classified according to the frequency of these fluctuations as radio waves; microwaves; infrared, visible, and UV waves; X-rays; and γ rays. The basic unit of electromagnetic radiation is the photon, which has no mass or electric charge and travels at the speed of light in a wavelike pattern. UV radiation is nonionizing radiation that is emitted by atoms when their electrons

descend from higher to lower energy states to yield photons of specific wavelengths and energy ranges. The emission of UV radiation from a mercury gas-filled light is illustrated in Figure 1. In this system, two high-voltage electrodes are placed in a mercury-filled chamber. The arc between the electrodes provides the energy for the electrons of the mercury atoms to enter a higher energy state, and photons are released as these electrons fall back to their basal low-energy states.
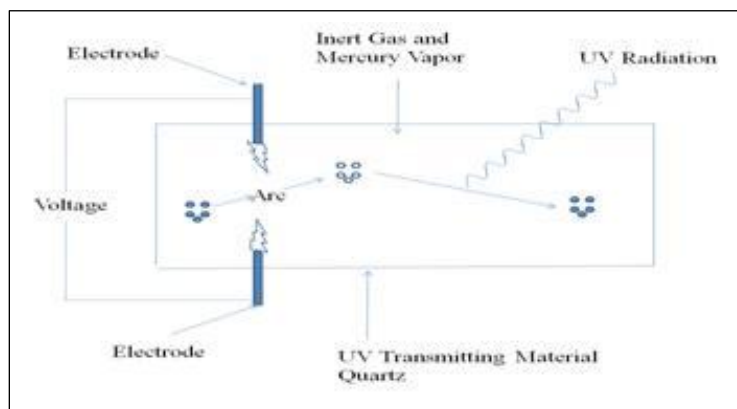

Figure 1. Release of UV radiation.

The UV wavelength region is between the visible and X-ray regions. The lower wavelength of the UV region is 10 nm, and the upper wavelength limit is 380 nm (or 400 nm, depending on the author), which is the beginning of the range that is visible to human eyes. As shown in the Table, the spectrum of UV light is divided into four regions: far UV or vacuum UV, UV-A, UV-B, and UV-C. These regions of the UV spectrum have markedly different effects on microorganisms. Far UV (<190 nm wavelength) radiation from the sun is absorbed by the atmosphere and does not reach the earth. For this reason, far UV radiation has little relevance to inactivation of organisms within the environment. Far UV wavelengths are highly deleterious to all life forms, but they are difficult to produce in the laboratory.

The precise boundaries between UV-A, UV-B, and UV-C often vary among authors and depend on whether the division is based on health effects or on the physical principles of the illumination sources. In general, from a health perspective, UV-B and UV-C wavelengths are defined to be germicidal, and UV-A radiation is considered to be nongermicidal. UV-A radiation includes the wavelengths between 320 and 380 (or 400) nm and is used in tanning lamps. UV-B radiation spans the wavelengths between 290 and 320 nm, and UV-C radiation contains the wavelengths between 190 and 290 nm. An alternate definition by the International Commission on Illumination (Vienna, Austria) defines UV-B radiation as having wavelengths between 280 and 315 nm.

The availability of low- and medium-pressure mercury lamps makes UV radiation relatively easy and inexpensive to obtain and use. Low-pressure mercury lamps are referred to as "monochromatic" in that they produce a narrow band of UV-C radiation at a wavelength of about 254 nm. Medium-pressure mercury lamps are described as "polychromatic" and produce a broader but flatter spectrum that extends from about 200 to 400 nm.

Table. Wavelength Ranges of UV Regions

Region	Wavelength, nm
Far or Vacuum UV	10–190
UV-C	190–290
UV-B	290–320
UV-A	320–380

Organisms are affected by UV exposure intensity (flux) and exposure time, and the multiplication of these is called the UV exposure (fluence). UV meters measure the flux (in watts per square meter), and this is multiplied by exposure time (seconds) to obtain the fluence (in joules per square meter). In addition to direct radiation from the sun or lamps, reflection from surfaces and particles in the environment is also important. Highly reflective environments such as white sand and fresh snow can significantly increase the UV irradiance in areas shaded from direct illumination and still disinfect organisms effectively (Ben-David and Sagripanti, 2010).

3.   SUNLIGHT

Increasing attention to epidemiology, biodefense, and public health has led increasing numbers of scientists to become interested in evaluating organism decontamination via sunlight exposure (Coohill and Sagripanti, 2009). The germicidal effect of solar radiation has been known for many years and was described as early as 1878 (Black and Veatch, 2010). The role of sunlight in human epidemics was demonstrated by an increase in hospital admissions for influenza during the burning season in Brazil, when smoke blocked the UV irradiation and reduced the inactivation of viruses in the air (Mims, 2005). Sunlight is known to kill organisms; however, conducting germicidal experiments using direct sunlight is challenging. Sunlight is difficult to control, and the risk of inadvertent contamination of samples is especially high outdoors.

Many factors affect the amount of UV radiation that reaches the earth's surface, including latitude, altitude, solar zenith angle (the angle between the sun and zenith during the day), the day of the year, and weather conditions (Lytle and Sagripanti, 2005). Environmental parameters such as total column ozone; the presence of clouds, pollutants, dusts, and aerosols; and reflection from the ground also affect radiation levels (Ben-David and Sagripanti, 2010). It is important to note that the atmospheric ozone layer absorbs radiation of wavelengths <290 nm, thus completely eliminating germicidal UV-C radiation and decreasing the amount of UV-B radiation that reaches the ground. For example, in regions with less atmospheric ozone, more UV-B radiation reaches the earth (Puskeppeleit et al., 1992; Lubin and Jensen, 1995). Additionally, the amount of UV-B radiation that reaches the earth is greater at high altitudes and low latitudes and during summer months, and it peaks at solar noon.

Researchers have used many methods to remove portions of the sun's spectrum to allow evaluation of the effects of UV-A and UV-B radiation separately and combined. For example, a single layer of Saran wrap (Dow Chemical Company, Midland, MI) allows

penetration of UV-A and UV-B radiation while protecting samples from environmental contaminants during exposure (Riesenman and Nicholson, 2000). In the same study, a 1.25 cm–thick glass plate was used to block UV-B and allow UV-A radiation to reach the samples. In addition to natural solar radiation, artificial lights that simulate sunlight are also used in experiments. Exposure to artificial lights is easier to control during experiments conducted inside the laboratory. Furthermore, medium-pressure mercury arc lamps that emit a UV spectrum in the range of 280 to 320 nm (peak at 302 nm) wavelengths can be used with filters that block wavelengths below 290 nm to yield the UV-B wavelengths (Riesenman and Nicholson, 2000).

Many researchers have measured the UV radiation doses received at different geographical locations. Heisler et al. (2004) measured the UV-B dose in Baltimore, MD for a 14 month period. When those measurements were compared to measurements from two other sites in Maryland, the results indicated that the UV radiation doses were similar at the three sites. The UV-B radiation was greatest during the June–July time period (when the solar elevation angle was also the highest) and depended on the annual stratospheric O_3 cycle. Results of this study indicated that the yearly average daily UV dose was 35,600 J/m^2. The maximum daily dose of 70,000 J/m^2 was observed in summer, and the minimum daily dose of 10,000 J/m^2 was observed in winter. On clear days, the maximum total UV-B irradiance in Baltimore ranged from about 3 W/m^2 (in June) to 0.6 W/m^2 (in December).

Hicke et al. (2008) studied radiation amounts in different parts of the country. The UV-B radiation-measuring instruments used in their study were designed to replicate the erythemal action spectrum. An erythemal action spectrum is a parameter that describes the relative effectiveness of different wavelengths of energy to produce a skin response. An action spectrum is used as a "weighting factor" for the UV spectrum to identify the actual biologically effective dose for a given result. Reports from this study indicated that over an eight-year period, the mean annual irradiance for Maine was 0.15 W/m^2 and for New Mexico was 0.35 W/m^2. The mean monthly irradiance for New Mexico was 0.5 W/m^2 in June and 0.18 W/m^2 in January, and for Maine was 0.25 W/m^2 in June and 0.05 W/m^2 in January. This shows that the amount of UV radiation varies throughout the United States, and it changes throughout the year.

For a given date, the flux does not change, on average, from year to year; therefore, previously published data can be reanalyzed using newly obtained flux measurements. For many sites in the United States, full daily UV-A and UV-B fluence measurements are obtained using broadband sensors or sensors tuned to certain wavelengths. This research is conducted by the UV-B Monitoring and Research Program (UVMRP), a data collection and research program of the U.S. Department of Agriculture headquartered at Colorado State University (Fort Collins, CO). The UVMRP website (http://UVB.nrel.colostate.edu) includes information about local UV-B and UV-A radiation that has been obtained since 1993.

4.   BACTERIAL ENDOSPORES

Organisms that form spores are among the life forms most resistant to disinfection and inactivation (Block, 2001). Endospores are highly resistant to a variety of stresses, such as toxic chemicals, desiccation, temperature and pressure extremes, and high doses of ionizing or UV radiation.

The high level of resistance of spores is related to their architecture. An illustration of the typical bacterial spore layers, including the exosporium, coats, outer membrane, cortex, germ cell wall, inner membrane, and core is shown in Figure 2. The outermost layer is the exosporium, which is a large, loose-fitting structure composed of proteins, including glycoproteins that are found only in some *Bacillus* species. The amount of exosporium varies with the species; for example, *Bacillus subtilis* has a thin layer of exosporium, if any. Inside the exosporium are the spore coats, which are complex structures formed by many proteins in multiple layers. The outer membrane, which is necessary for spore formation, lies under the spore coat. Located within the outer membrane, the cortex is essential for reduction of the water content in the spore core and formation of a dormant spore. The cortex is degraded in spore germination, and this degradation is required for spore core expansion and subsequent outgrowth to occur. The germ cell wall (composed of peptidoglycan) under the cortex becomes the cell wall of the outgrowing spore. The inner spore membrane serves as a strong permeability barrier to chemicals that damage spore DNA. The spore core contains DNA, enzymes, ribosomes, and transfer RNA.
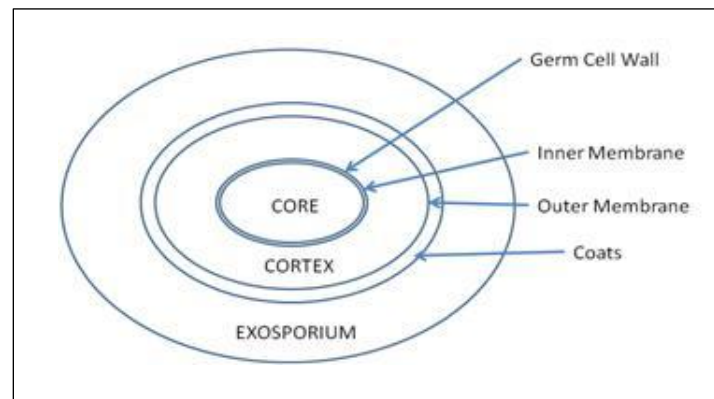
Figure 2. Spore structure. (Data from Setlow, 2003.)

Microorganisms use several mechanisms to protect themselves from environmental stresses such as UV radiation. These include:

1. Nucleocapsids and cytoplasm contain UV radiation-absorbing proteins.

2. The majority of bacterial spores identified at high altitudes contain pigments such as carotenoids and melanins that scavenge the free radicals produced by oxidative damage caused by UV radiation (Moeller et al., 2005).

3. Organisms can contain a biochemical pathway for repairing the DNA damage caused by UV radiation.

4. Organisms are protected by thick spore protein coats that consist of inner and outer coat layers.

5. The core's low water content keeps DNA in a specific conformation (the A form) that is more resistant to UV radiation (Setlow, 2006).

Bacillus endospores are the best characterized species among spore-forming bacteria and are used as a model system for studying the resistance of bacterial endospores to environmental extremes. Studies have shown that *B. anthracis* Sterne spores and *B. subtilis* spores have identical UV inactivation kinetics; therefore, *B. subtilis* strains could reliably be used as a biodosimetry model for the UV inactivation of *B. anthracis* spores (Nicholson and Galeano, 2003; Menetrez et. al., 2006). However, various researchers identified different inactivation kinetics for the same organisms. This may be due to a lack of consistent exposure techniques between laboratories, varying spore concentrations in the liquid samples (mixed or not mixed), the quality and state of the spores that were used in the studies, and the proportion of vegetative bacteria in the samples. The differences identified between some species pertained to plasmid content, proteins, and the presence and thickness of spore coats.

5.   VEGETATIVE BACTERIA

Vegetative bacteria continuously grow and reproduce. Unlike endospores, vegetative bacteria lack spore coats and an exosporium; however, they have a cell wall that is composed of peptidoglycan (protein and sugar). Vegetative bacteria can be classified as either Gram positive or Gram negative, depending on whether the cells retain crystal violet dye during the Gram-staining protocol. Gram-positive bacteria have a thick peptidoglycan layer within their walls, which also contain modified alcohols called teichoic acids (Kratz, 2005). Some bacteria, such as *Mycobacterium tuberculosis*, have a waxy outer layer that is resistant to disinfectants. Gram-negative bacteria have a thin peptidoglycan layer within their walls and an additional outside layer called the outer membrane. The outer membrane is composed of phospholipids, lipopolysaccharides, lipoproteins, and channel proteins. Some bacteria do not have Gram-positive or Gram-negative cell walls. The nuclear material of vegetative bacteria is the target site for damage by UV radiation. The nuclear material is located inside the fluid-filled cytoplasm, which is within the plasma membrane.

6.   VIRUSES

Viruses, which are among the smallest infectious agents, exist in the boundary between living organisms and chemical entities. Viruses are composed of nucleic acids surrounded by a protein coat called the capsid. Different viruses can infect and then replicate within a variety of host cells. Some viruses have an additional layer surrounding the capsid, called the envelope, that is made of phospholipids, proteins, and carbohydrates. The envelope is taken from the host as the virus exits the host cell. The nucleic acids in a virus can be DNA or RNA and may be double- or single-stranded, and some viruses contain both DNA and RNA.

Many viruses (more than 90) can cause disease in humans (*Fields Virology*, 1990, 2001). Because of their apparent fragility and sensitivity to salts, solvents, and pressure, viruses might be expected to be more sensitive than bacteria to other environmental effects. However, recent observations and experimental measurements indicate that viruses can reach further into the environment than previously expected. In this regard, an information leak from the former Soviet Union reported an accidental infection of naval personnel 11 miles offshore from a smallpox testing site (Tucker and Zilinskas, 2003). This indicated that viruses can survive for long time periods and over great distances outdoors and are not as vulnerable as previously thought.

Lytle and Sagripanti (2005) reviewed published data on virus sensitivity to UV light and devised an approach for estimating survival of various viruses after their release at any location and at any time of the year. Ben-David and Sagripanti (2010) modeled the use of solar UV radiation to inactivate viruses aerosolized in the atmosphere. They estimated that a full day of sun exposure would decrease the infectivity of UV-sensitive viruses on average by 3 $\log_{10}$. Similar calculations were used by public agencies to estimate viral persistence in the environment during recent influenza epidemics (Sagripanti and Lytle, 2007).

Although mammalian viruses are the causative agents of human epidemics, few have been used in UV experiments and other survival studies because of safety concerns. Bacteriophages are often studied as surrogates to infectious viruses; however, phages differ greatly in morphology, composition, and UV resistance when compared with mammalian viruses of interest in public health and biodefense (Lytle and Sagripanti, 2005). For these reasons, bacteriophages are not useful surrogates for studying the sensitivity of mammalian viruses to UV light.

7.   CELL DAMAGE DUE TO UV RADIATION

DNA is the most sensitive target of an organism, and UV light chemically modifies the genetic material of microorganisms (Lytle and Sagripanti, 2005). Unsaturated bonds present in biological molecules such as coenzymes, hormones, and electron carriers may also be susceptible to UV damage.

Nucleic acids have absorption peaks at 200 and 265 nm. The 200 nm radiation is absorbed by molecules of ribose and phosphate in the DNA backbone, and the 265 nm radiation is absorbed by the nucleotide bases thymine, adenine, cytosine, and guanine (Kowalski, 2009). Thus, among the UV radiation regions, UV-C radiation (254 nm, close to the maximal absorption of DNA) inactivates organisms most effectively, UV-A radiation (320–380 nm) inactivates organisms least effectively, and the effectiveness of UV-B radiation (290–320 nm) falls in the middle. Sensitivity to UV light correlates inversely with the size (or molecular weight) of the genome, such that smaller DNA molecules are more severely affected by UV radiation (Lytle and Sagripanti, 2005).

UV radiation can cause crosslinks to form between two adjacent thymine bases. The result is the formation of stable thymine dimers that inhibit replication and inactivate bacteria and DNA viruses (Masschelein and Rice, 2002). In addition, thymine forms crosslinks with thymine in adjacent DNA strands and proteins; this inactivates the DNA and negatively affects reproduction. Crosslinks can also occur between cytosine and guanine, but more energy is required due to the three hydrogen bonds between them. Other dimers formed after UV exposure include cytosine dimers, cytosine-thymine dimers, uracil dimers, uracil-thymine dimers, and uracil-cytosine dimers. Dimer formation occurs mainly after UV-A exposure, whereas lesions of other types occur due to UV-B and UV-C exposure. Fewer dimers are formed in the hydrated state, because it is less compact and the nucleotides are relatively far apart (Schreier et al., 2007).

Uracil takes the place of thymine in RNA. Uracil dimers are formed after UV exposure of RNA, but not to the extent that thymine dimers are formed in DNA. RNA also covalently links with proteins in response to UV irradiation. The pyrimidine-pyrimidone (6-4) series of lesions (called 6-4 photoproducts) also occurs due to UV exposure.

Because it has more structural stability, double-strand RNA is more stable after UV exposure than single-strand RNA (Rauth, 1965; Becker and Wang, 1989). Parallel findings in DNA viruses indicate that double-strand DNA viruses are also more resistant than single-strand DNA viruses (Tseng and Li, 2007). In addition, DNA viruses are more resistant than RNA viruses with the same number of strands (double or single).

Similar to nucleotide crosslinks, nucleotides can also bind with water in response to UV exposure in a process called photohydration. Photohydration reactions cause cytosine and uracil to bond with water molecules; however, this does not occur with thymine. Unlike DNA and RNA damage, photohydration is independent of wavelength size (Cerutti et al., 1969; Remsen et al., 1970).

8.   PHOTOREPAIR

Many environmental bacteria live in balance between UV damage and repair. The repair mechanisms start immediately after sunlight irradiation and continue into the night, with the organisms needing to be fully repaired the next morning. The repair that occurs during the night is called dark repair. The use of light energy for organism repair after UV damage is called photorepair, photorecovery, or photoreactivation (Peccia and Hernandez, 2001; Xue and Nicholson, 1996). Photorepair activities show an initial peak in the UV-A region (320–380 nm). In addition, visible light (380–750 nm) is also used by cells undergoing photorepair. The degree of photoreactivation of vegetative bacteria depends on the particular species and strain. Photorepair does not occur while bacteria that can do so are in the spore stage, but it does occur during and after spore germination.

Three primary repair processes are used by organisms in response to UV radiation. In the first process, called the excision repair process or the cut-and-patch mechanism, a series of cellular enzymes replaces thymidine dimers with undimerized thymine. The second process occurs in some spores and vegetative bacteria that can use photons in visible and UV light to reverse DNA damage by monomerizing dimers. The third repair mechanism rescues cells from severe DNA damage: polymerization around DNA damage is followed by excision of the damaged areas and normal DNA synthesis. However, this process may lead to mutation due to the addition of extra bases (Coohill and Sagripanti, 2008, Hanawalt, 1989). Photorepair processes are optimized at different temperatures, depending on the enzymes used by particular cells. Humidity also affects the photorepair process; exposure to high relative humidity (RH) environments generally supports photoreactivation by facilitating overall cell function.

Repair intensity depends on the amount of damage sustained and the biological organization of the microorganism. The repair mechanism involves the production of enzyme-substrate complexes at the DNA lesion site, where light energy is absorbed for the repair. Photorepair fixes the damages that result from narrow-band UV-C radiation (254 nm) exposure. Radiation produced by broad-spectrum UV lamps damages the enzymes and other microbial constituents, and photorepair processes are unable to fix these damages.

9. TYPICAL SURVIVAL CURVE FOR UV EXPOSURE

The kill effect of UV radiation can be quantified by several parameters, such as the doses required to kill 90 and 99% of the present organisms (D_{90} and D_{99}, respectively) and the dose required for 37% survival of the organisms (D_{37} or e^{-1}). D_{90} and D_{99} are often indicated as F_{-1log} and F_{-2log}, respectively (King et al., 2011). D_{37} corresponds to the amount of radiation required to produce, on average, one lethal hit per organism (Lytle and Sagripanti, 2005).

A curve describing the survival of organisms after UV exposure has been modeled to incorporate different complexities, such as how the organism is damaged and how it repairs the damage. The disinfection curve shown in Figure 3 reflects a simple exponential eq 1. This is a first-order decay model that provides relatively accurate values for D_{90} and D_{99}. Higher values for rate constants (or slope) indicate fast disinfection, and lower values indicate slow disinfection:

$$S = e^{-kD} \tag{1}$$

where S is the survival fraction (the number of organisms surviving radiation divided by the number of organisms before irradiation), k is the UV rate constant (generally in m^2/J), and D is the dose or fluence (generally in J/m^2).
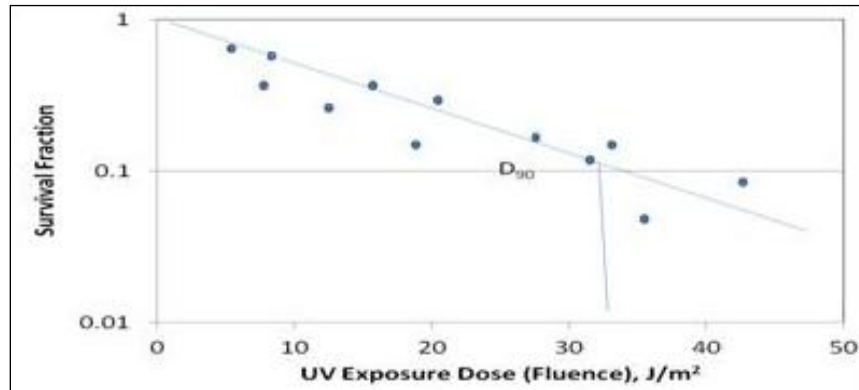
Figure 3. Example of a disinfection or decay curve that shows first-order decay.

Often, a tiny fraction (sometimes <1%, but as high as 40–50% when protected by media or pigments) of the organisms exhibits a higher level of resistance and a significantly lower rate constant. In these cases, a fast decay is observed, which is attributable to the susceptible population; this is followed by a slower decay that corresponds to the apparently resistant population. If the resistant population is on the order of 1% or less, it can be identified graphically after the D_{99} value has been reached. In this two-stage decay curve (shown in Figure 4), survival is described by

$$S = (1 - f)e^{-Dk_1} + fe^{-Dk_2} \tag{2}$$

where f is the resistant fraction, k_1 is the first-stage rate constant, and k_2 is the second-stage rate constant.

Figure 4. Example of disinfection curve that shows the first- and second-stage decays.

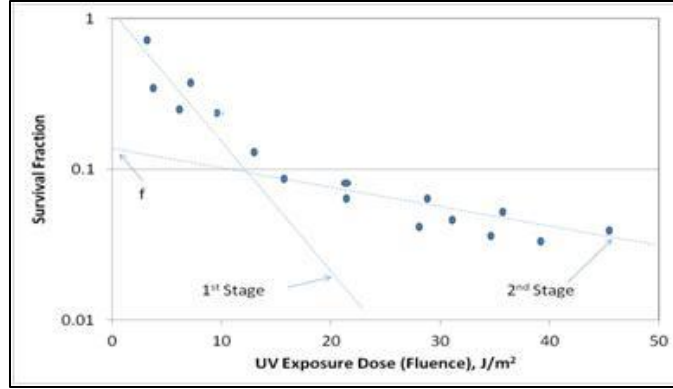The early part of the curve could also have a shoulder region, where there is no permanent damage. The bacteria can repair the damage caused by low levels of UV exposure. This has been experimentally shown by a lack of change in culturable bacteria concentrations with increasing exposure to certain levels of UV radiation (reviewed in Coohill and Sagripanti, 2008). The length of the shoulder region reflects the ability of the organisms to withstand and repair damage, and it is dependent upon the particular organism and the assay sensitivity. The shoulder region is followed by the first-stage linear region on a $\log_{10}$-linear graph, as shown in Figure 5 (Coohill and Sagripanti, 2008). The multihit model (Kowalski, 2009) shows the survival curve that includes the shoulder and linear portion as

$$S(t) \; = \; 1 - (1 - e^{-kD})^n \tag{3}$$

where k is the rate constant (the slope of the linear portion of the curve), and n is the shoulder constant that is equal to the y-axis intercept. Equations used to describe microbial decay that include a shoulder followed by two (fast and slow) rate decays also exist, but are not shown here.
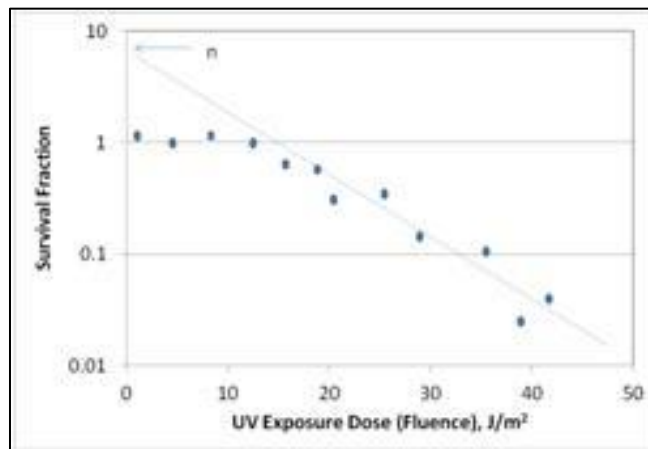

Figure 5. Example of disinfection or decay curve that shows the shoulder and first-stage decay regions.

As shown in Figure 4, the decay curves can end in a tail that indicates the presence of a resistant population. There are also other reasons for the appearance of tailing. For example, tailing often occurs in the decay curves from experiments conducted with bacteria on agar surfaces. Agar surfaces have microscopic pits and fissures. Bacteria inside these microcrevices are exposed to less or no UV radiation, and therefore appear to be resistant. If one bacterium in a thousand was shielded, then the linear portion of the decay curve would end at the $-3 \log_{10}$ level, and tailing would be evident after that point. For bacteria exposed on filter paper, the resulting decay curves are determined by the filter paper structure and moisture content. To reduce these surface effects, some researchers have irradiated the bacteria while stirring them in dilute solutions in an attempt to expose all of the organisms to comparable levels of UV radiation (Moeller, et al., 2005). For these experiments, the medium must be transparent, and cell density must be sufficiently low such that no shadow effects occur between cells in the center of the container and those closer to the container walls.

10.   THE UV RATE CONSTANT

When organisms are exposed to UV light, they decay as a function of dose (fluence), where the slope of the logarithmic decay curve is defined as the rate constant, k. The rate constant is affected by RH, temperature, DNA conformation (A or B), irradiance level, photoreactivation, type of UV lamp used, dose range used in the test, culture media and method, and specific strain of organism used. As discussed in the previous section, for various organisms, the resulting decay curves have different shapes, with the linear portions resulting in different rate constants. It is difficult to make comparisons between organisms that have different shapes of decay. Therefore, the D_{90} value is frequently used to compare different organisms, and a rate constant (known as the UV susceptibility, with units of square meters per joule) is calculated as

$$D_{90} = \frac{-\ln(1-0.9)}{k} = \frac{2.3}{k} \tag{4}$$

This equation does not describe the actual shape of the decay curve that may have a shoulder and one or two linear portions; therefore, D_{90} is used more often to compare between studies or organisms. The dose needed to reduce the microbial population to 10% of the original amount is indicated as T_1, and the fluence that reduces the microbial load by $4 \log_{10}$ is indicated as T_4. Alternatively, a parameter derived from quantum mechanics and statistical damage has been used by scientists to characterize microbial sensitivity to UV radiation. The D_{37} value is equal to the reciprocal of the slope and corresponds to the UV fluence that produces, on average, one lethal hit to the organisms, to result in a survival rate of 37%. D_{37} can be calculated by dividing the fluence that inactivates a $1 \log_{10}$ virus load (as obtained from the linear portion of the graphs) by 2.3 (the natural logarithmic base). A lower value for the D_{37} value indicates a higher sensitivity to inactivation by UV radiation.

11.   RH AND TEMPERATURE EFFECTS

The effect of RH levels on microbial susceptibility to UV radiation has been studied for many years; however, a clear consensus has not been achieved. As mentioned in the previous section, DNA exists in A and B conformations. The conformations change in response to varying RH levels. The A conformation occurs during low RH conditions, when water molecules are desorbed from DNA due to the hydrophobic nature of the tighter internal channels. During high RH conditions, the DNA hydrates into the B conformation. Most bacteria tend to exhibit decreased susceptibility when exposed to high RH levels, but some bacteria respond with the opposite effect or no effect at all, and viruses respond with mixed effects. In studies of *Escherichia coli*, *Mycobacterium parafortuitum*, and *Staphylococcus epidermis*, low susceptibility was identified during exposure to high RH conditions (Kowalski, 2009). Xu et al. (2005) reported similar results in experiments in which *M. parafortuitum* cells were aerosolized to determine the effectiveness of upper-room air UV germicidal irradiation. McDevitt et al. (2007) showed that the susceptibility of vaccinia virus increased with decreasing RH. In addition, Tseng and Li (2007) evaluated the survival fraction of four viruses at 55 and 85% RH levels, and the results indicated that susceptibility was increased at lower RH levels. Similar effects were observed in a study by Lin and Li (2002), in which microorganism susceptibility at 80% RH was lower than at 50% RH. The organisms used in this test were *E. coli* cells, *B. subtilis* spores, *Candida famata* var. *flareri* cells, and *Penicillium citrinum* spores. A few studies indicated that susceptibility was high for organisms in high RH environments. For example, for some strains of *Serratia marcescens*, disinfection was increased at higher RH levels (Kowalski, 2009).

Kowalski (2009) reported that the D_{90} value for inactivating organisms in high-RH air was fivefold higher than for organisms in low-RH air. In that same report, the D_{90} value for inactivating organisms in water was twofold higher than for organisms in high-RH air.

In any case, the apparently contradictory reports regarding changes attributable to RH describe only modest effects on the susceptibility of organisms to UV radiation. For example, normal environmental RH changes (50–70%) only affected survival of the influenza virus by 9%, and extreme RH changes (15–90%) affected the viruses by 12.5-fold (Hemmes et al., 1960). An infected patient's nasal secretions can contain 10^7 viral particles per milliliter (Couch, 1995), and an increase in RH may reduce the viable particle concentration to 10^6 particles per milliliter. This reduction is not significant when considered in the context of preventing disease transmission. In comparison, the effects of solar radiation or indoor UV lamps on the inactivation of microorganisms outdoors and indoors are substantial.

Temperature also influences the effects of UV radiation. Higher temperatures can cause structural changes in cell membrane phospholipids, proteins, and DNA. Ko et al. (2002) reported that UV effectiveness was significantly increased when temperature was increased from 4 to 25 °C. UV radiation is likely to cause mutagenic effects at high temperatures, where DNA becomes more flexible due to a more active metabolism.

12.   BACTERIA CLUSTERS

        The majority of the previous studies intended to establish the effects of germicidal UV lights or sunlight were conducted using single spores on surfaces and in air (King et al., 2011; Foarde et al., 2006; reviewed in Coohill and Sagripanti, 2009). However, the outer layers of spores in a cluster could protect the organisms in the center. The killing effect of UV light on all the organisms in the cluster may be underestimated by data obtained from single spore dispersion tests.

        Clumping also shields bacteria in the center from UV effects and can therefore affect survival curves. On the basis of previous calculations for irradiation with 254 nm wavelength, 61% of the incident light will be transmitted through one organism (Coohill, 1986). Similarly, two organisms shielding a third organism will only allow 37% of the incident UV light to reach the third organism. Therefore, clumping can protect the bacteria in the center from UV light and thereby cause the tailing observed in the decay curves from some experiments.

13.   DISINFECTION OF ROOMS USING UV-C RADIATION

        UV lamps generating radiation of 254 nm wavelength (UV-C) have been proposed as a supplemental germicidal control in healthcare facilities (TB Infection-Control Guidelines Work Group, 1994) because of the associated low costs, ease of application, and potential efficiency. UV-C lamps can be used in enclosed mechanical ventilation system ducts and locally recirculating units as well as in open configurations in rooms.

        In several studies (Rutala et al., 2010; Xu et al., 2003), a reduction in bacterial spore and vegetative cell numbers occurred in response to upper-room air UV irradiation. As expected, UV-C lights installed to irradiate upper-room air were reported to be more effective at inactivating vegetative bacteria than bacterial spores (Miller and Macher, 2000). In one of the first studies to demonstrate the benefits of germicidal UV lights, the number of influenza infections in a veterans' hospital was shown to be reduced when compared to infections in nearby, nonirradiated hospital rooms (McLean, 1956). The U.S. Center for Disease Control and Prevention (CDC) recommends that hospital isolation rooms be ventilated with at least 12 clean, outside air changes each hour (Siegel et al., 2007). In addition, the general rule for the number of UV lightbulbs in a room is that one 30 W fixture should be used for every 18 m^2 of floor area or for every seven people in the room. In a study by Xu et al. (2005) that included fourfold higher UV wattage than is recommended by CDC guidelines, it was shown that UV irradiation that is uniformly distributed throughout a room is more effective than UV radiation of nonuniform distribution.

        In a study conducted by Menzies et al. (2003), it was shown that UV-C lights installed in office ventilation systems reduced microbial and endotoxin concentrations on irradiated surfaces within the ventilation systems by 99% (2 $\log_{10}$) and also reduced respiratory and musculoskeletal symptoms in office personnel. Ko et al. (2002) showed that UV germicidal irradiation in an upper room significantly reduced culturable bacteria concentrations in a lower room. A study conducted in a test chamber (King et al., 2011) showed that bacteria culturability was reduced in response to UV-C radiation exposure. These findings indicate that UV-C irradiation can contribute to the control of contagious disease.

14.   SUNLIGHT EXPOSURE STUDIES

In previous studies, the sun as well as artificial light sources designed to simulate the sunlight spectrum (Munakata, 1999; Munakata and Kazadzis et al., 2000; Munakata and Makita et al., 2000) were used to expose organisms to direct and indirect radiation. The effects of direct and indirect radiation must be considered to completely explain the effects of UV radiation on organisms.

Analysis of sunlight-exposure studies indicated that the UV sensitivity of *B. anthracis* Sterne spores was similar to that of *B. subtilis* spores (strain SN624, ATCC 6633), which suggests that *B. subtilis* can be used as a surrogate for *B. anthracis* Sterne in UV decontamination studies (Nicholson and Galeano, 2003). Also a carotene-like pigment found in *Bacillus atrophaeus* DSM 675 spores at high altitudes affects the resistance of the spores to environmental UV radiation (Moeller et al., 2005).

UV doses measured by the U.S. Department of Agriculture were used to determine virus kill rates from sunlight exposure (Sagripanti and Lytle, 2007; Lytle and Sagripanti, 2005). The flux at each wavelength in the UV-B region was multiplied by an appropriate normalized action spectrum value that was based on the 254 nm kill effect. It was determined that the germicidal activity of sunlight peaks at 300–305 nm, with the solar flux between these wavelengths accounting for 75% of the total effective germicidal solar flux (Sagripanti and Lytle, 2007). Thus, a UV dosimeter that is sensitive between 295 and 305 nm provides a usable correlation between solar light dose and its germicidal effect (Coohill and Sagripanti, 2009).

For reasons of convenience, artificial sunlight is preferred for testing environmental solar effects (U.S. Environmental Protection Agency, 2010). Beebe et al. (1962) exposed *B. anthracis* Ames to 600 μW/cm^2 (1 W/m^2) of artificial sunlight using General Electric (Schenectady, NY) type RS sunlamps with wavelengths <305 nm removed. Mice were exposed to nonirradiated and irradiated *B. anthracis* spores to determine the effect of simulated solar UV on virulence. In addition to experiments, mathematical models to predict virus inactivation by solar radiation have been developed by Lytle and Sagripanti (2005) and Ben-David and Sagripanti (2010). These authors have predicted the inactivation of smallpox virus as well as Ebola, Marburg, Crimean-Congo, Junin, and other hemorrhagic viruses and Venezuelan equine encephalitis in different parts of the world. Using calculations allows researchers to predict the survival of organisms in outdoor settings while avoiding the dangers of working with infectious agents.

15.   TESTING CONSIDERATIONS

In many of the previous studies on the effects of UV radiation, not all variables were controlled, nor was all the information necessary for duplicating the experiments and confirming the findings reported. Variables that should be reported in UV-C and solar exposure studies have been reviewed (Coohill and Sagripanti, 2009) and include temperature, RH, moisture state of the particle (wet or dry), surface conditions (if microbes are deposited on surfaces), dosimetry, environmental conditions (date, time of day, altitude, latitude), organism stage (exponential or stationary phase), amount of debris present, and ratio of vegetative-to-spore

bacteria, as well as any other relevant chemical or biological conditions. Biological parameters such as the ability of the organism to repair, its life cycle stage, sample temperature, and the UV spectrum of the radiation are among the most critical variables to be specified if reproducibility is intended.

Previous studies have shown that the type of microbial damage that occurs after solar UV irradiation depends on wavelength. For example, the biocidal effectiveness of the 300 nm wavelength is 100-fold higher than that of the 320 nm wavelength. In laboratory-based experiments, a relatively narrow range of wavelengths is generally used to mimic solar radiation. A disadvantage of using the narrow-range exposure method is that the solar spectrum has been reported to produce biological effects that can differ by an order of magnitude from those of laboratory-based, monochromatic radiation studies. In addition, UV-A and visible radiation can support photorepair of the damage caused by other wavelengths, and this effect would not be observed in the exposure of microbes to a narrow range of wavelengths.

Sunlight exposure time and duration are important parameters for predicting radiation wavelength and dose to organisms. The greatest solar damage occurs at solar noon, and less damage occurs on either side of noon due to the decrease in UV-B flux. Therefore, sun exposure time must be recorded, and dosimetry must be measured accurately.

Unexplained differences between the effects of artificial sources and sunlight on microbial inactivation have been suggested. Formation of different photoproducts were reported in response to exposure to UV-A, UV-B, and UV-C radiation (Nicholson et al., 2002). Therefore, extreme care must be exercised when selecting exposure wavelengths and matching the proper dosimeter with a wavelength or spectrum response that accurately reflects the dose received by organisms during testing.

Three distinct methods are used by researchers to determine the effects of UV radiation on microorganisms on surfaces, in liquids, or in aerosols. In general, the disinfection of organisms on surfaces is studied by exposing organisms on agar plates. The agar plate method only allows organisms to be exposed to UV light on one side. Alternatively, organisms in liquid or air suspensions are exposed to UV light from all directions. When organisms are exposed to an identical radiation source under any of the three physical conditions (surface, liquid, or aerosol) different fluencies are required to attain comparable damage.

The sensitivity to UV of bacterial spores is the aim of many studies. The quality and state of the spores in a sample affect their response to UV irradiation. The ratio of vegetative bacteria to spores in a bacterial preparation strongly affects the response of that preparation to UV irradiation because spores are 5- to 10-fold more resistant to UV radiation than are vegetative bacteria (Coohill and Sagripanti, 2008). The physical condition of the sample also affects inactivation because organisms in liquid or on agar plates have layers of water around them, which may offer a level of protection that is not experienced by organisms on dry surfaces or suspended in air.

During solar exposure, spore inactivation is independent of temperature; however, vegetative bacteria are more resistant at higher temperatures because vegetative cells, as opposed to spores, can undergo photoreactivation to repair the suffered damage. When it is desirable to reduce or eliminate the photoreactivation, vegetative bacteria are exposed to sunlight at or near

15

0 °C. Furthermore, spores can be in the dormant state for many weeks after exposure, but vegetative bacteria must be cultured immediately after exposure. Repair-deficient mutants of vegetative bacteria are used in some studies to determine inactivation kinetics, but these tests occur rapidly and require shorter exposure times that are difficult to measure accurately.

16.   TEST METHODOLOGY IN OUR LABORATORY

We have developed a methodology for determining the disinfection efficiencies of UV-C radiation and simulated sunlight on organisms suspended in air. We have tested single spores and spore clusters of different sizes. A similar methodology was used to study the effect of UV light on vegetative bacteria. Organisms deposited on membrane filters and agar surfaces were used to determine the decontamination effectiveness against organisms on surfaces. Chambers filled with bioaerosols were used for aerosol testing.

17.   EQUIPMENT USED DURING TESTING

For the aerosol disinfection studies, a Collison nebulizer (BGI, Inc., Waltham, MA) was used to generate particles containing single organisms. For aerosol testing, a Sono-Tek aerosol generator (Milton, NY) was used to produce bioaerosol clusters. Each instrument and test chamber is briefly described in the following sections. Examples of single spores and clusters generated during testing are shown in Figure 6.
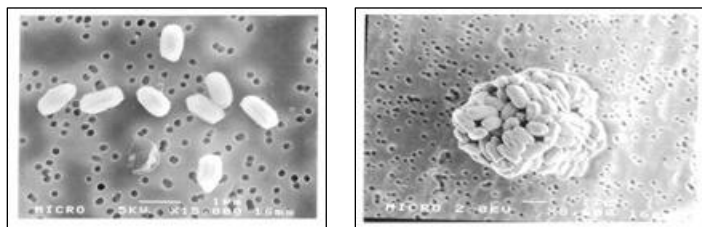

Figure 6. Single (left) and approximately 4 μm clustered (right) bacterial spores.

18.   COLLISON NEBULIZER

Collison nebulizers (Figure 7) with 4, 6, 24, and 36 jets were used at ECBC to generate single spore particles for aerosol testing. Test organisms were added to deionized water, and this solution was used in the nebulizer. To minimize the possibility of doublet and triplet organisms occurring in each aerosol particle, relatively low organism concentrations were used. The Collison nebulizer was connected to a compressed air source. The air exited at high velocity from small holes inside the nebulizer. The low pressure created in the exit region caused the water-organism solution to be drawn from the bottom of the nebulizer through a second tube (in accordance with the Bernoulli effect). The liquid exited the tube as a thin filament. As it accelerated in the airstream, the filament stretched until it broke into droplets. The spray stream was directed onto a wall. Larger droplets impacted the wall and were removed from the air.

Figure 7. A Collison nebulizer.

19.   SONO-TEK AEROSOL GENERATOR

Sono-Tek ultrasonic atomizing nozzles, like the one shown in Figure 8, were used to produce droplets of the liquid and material mixtures suspended in liquids that were then dried to yield a dry aerosol of residual material particles. In biological experiments, the aerosolized mixture was composed of organisms suspended in water. Final particle size was determined from the material concentration in the feed liquid and the characteristic size of the Sono-Tek droplets. The rate of aerosol generation was dependent upon the rate at which the fluid mixture was fed to the Sono-Tek nozzle. Five models of Sono-Tek aerosol generators are available to produce particles with mean diameters ranging from 23 to 70 μm (at 120–25 kHz operating frequencies) and flow rates from a few microliters per second to about 6 gallons per hour. We used the 25 kHz model, fed by a peristaltic pump, and the 120 kHz microbore model, fed by a syringe pump. These were used to create controllable aerosol concentrations from about 500 to 50,000 particles per liter of air inside a chamber.

Figure 8. Sono-Tek aerosol generators.

20.   AEROSOL EXPOSURE TEST METHODOLOGY

        Figure 9 is an illustration of our aerosol exposure chamber, which has dimensions
of $36 \times 17 \times 14$ in. and a volume of 0.14 m^3. Single spore particles were generated using the
Collison nebulizer, and bioclusters were generated using the Sono-Tek ultrasonic atomizing
nozzles. After the aerosol was generated, the chamber air was mixed to produce a uniform
aerosol concentration. A baseline air sample was obtained on gel filters before the UV exposure
occurred. The organisms were then exposed to UV light for different lengths of time. Air
samples were collected on gel filters at the end of each exposure. Control samples were collected
in a similar fashion, but without the UV light exposures (while the UV light was turned off). The
amount of kill due to UV exposure was determined by comparing the baseline samples with the
UV light-exposed and control samples (King et al., 2011).
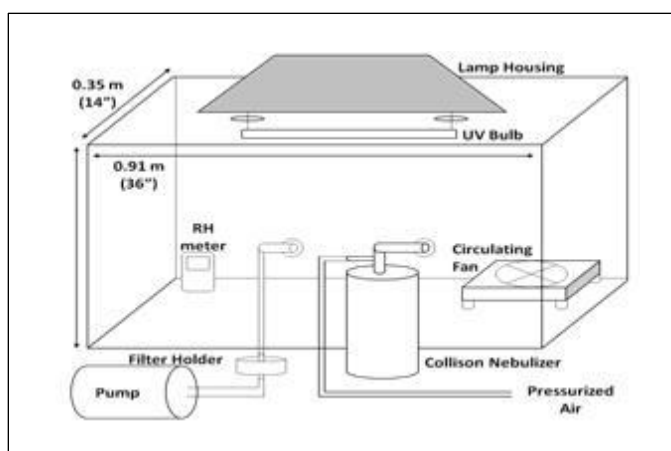

Figure 9. Aerosol exposure chamber.

21.   DISCUSSION

        The unintentional release of bioaerosols from infected patients is a concern to
public health, and the intentional or accidental release of aerosolized microorganisms is a
concern in biodefense. Dilution and directed ventilation reduce risk by decreasing microbial
concentration or removing organisms, but these methods do not kill germs. Air filtration
technology is used to reduce the number of infectious organisms in a room. High-efficiency
particulate air (HEPA) filtration can remove >99.99% of the airborne particles that arrive at the
filter media, but implementation of HEPA filtration often requires costly engineering
modifications. Among the air- and surface-disinfection technologies available, one with the
widest potential application could be UV-C disinfection because it is relatively easy to use and
highly effective. In addition, UV-C lamps can be installed in buildings for a fraction of the cost
of HEPA filtration systems. As indicated by the research summarized in this report, care is
needed to obtain accurate measures of microbial sensitivity to UV radiation. However, once
accurate microbial sensitivity measurements are available, researchers and engineers can use this
data in a wide range of applications. The use of UV-C radiation in a predictable fashion to
inactivate germs suspended in aerosols or deposited onto surfaces should allow for the

development of precise and effective engineering solutions for protecting health and life. Understanding the effect of natural sunlight on airborne germs should assist in understanding and thus predicting microbial dissemination and its role in natural or manmade epidemics.

Blank

REFERENCES

Becker, M.M.; Wang, Z. B–A. Transitions within a 5 S Ribosomal RNA Gene are Highly Sequence-Specific. *J. Biol. Chem.* **1989**, *264*(7), 4163–4167.

Beebe, J.M.; Dorsey, E.L.; Guse, D.G.; Hunt, G.R. *Stability and Virulence Relationships of Air-Borne* Bacillus anthracis *Spores Under Stress of Light and Humidity.* Technical Memorandum 18; U.S. Army Biological Laboratories: Fort Detrick, MD, 1962.

Ben-David, A.; Sagripanti, J.-L. A Model for Inactivation of Microbes Suspended in the Atmosphere by Solar Ultraviolet Radiation. *Photochem. Photobiol.* **2010**, *86*, 895–908.

Black and Veatch Corporation. *White's Handbook of Chlorination and Alternative Disinfectants*, 5th ed.; John Wiley & Sons: Hoboken, NJ, 2010.

Block, S.S. *Disinfection, Sterilization, and Preservation*, 5th ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2001.

Cerutti, P.A.; Miller, N.; Pleiss, M.G.; Remsen, J.F.; Ramsay, W.J. Photohydration of Uridine in the RNA of Coliphage R17. I. Reductive Assay for Uridine Photohydration. *Biochemistry* **1969**, *64*, 731–738.

Coohill, T.P. Virus Cell Interactions as Probes for Vacuum Ultraviolet Radiation Damage and Repair. *Photochem. Photobiol*. **1986**, *44*, 359–363.

Coohill, T.P.; Sagripanti J.-L. Overview of the Inactivation by 254 nm Ultraviolet Radiation of Bacteria with Particular Relevance of Biodefense. *Photochem. Photobiol.* **2008**, *84*,1084–1090.

Coohill, T.P;. Sagripanti, J.-L. Review: Bacterial Inactivation by Solar Ultraviolet Radiation Compared with Sensitivity to 254 nm Radiation. *Photochem. Photobiol.* **2009**, *85*, 1043–1052.

Couch, R.B. Orthomyxoviruses. In: *Medical Microbiology,* 4th ed. University of Texas Medical Branch: Galveston, TX, 1995, pp 1–22.

Fields Virology; Fields, B.N.; Knipe, D.M., Eds.; 2nd ed.; Raven Press: New York, 1990.

Fields Virology; Knipe, D.M.; Howley, P.M., Eds.; 4th ed.; Lippincott Williams and Wilkins: Philadelphia, PA, 2001.

Foarde, K.; Franke, D.; Webber, T.; Hanley, J.; Owen, K. *Biological Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Systems.* Technology Evaluation Report. U.S. Environmental Protection Agency, Office of Research and Development, National Homeland Security Research Center, 2006.

Giese, A.C. *Living with Our Sun's Ultraviolet Rays*. Plenum Press: New York, 1976.

Hanawalt, P.C. Concepts and Models for DNA Repair: From *Escherichia coli* to Mammalian Cells. *Environ. Mol. Mutagen.* **1989**, *14*(16), 90–98.

Heisler, G.M.; Grant, R.H.; Gao, W.; Slusser, J.R. Solar Ultraviolet-B Radiation in Urban Environments: The Case of Baltimore, Maryland. *Photochem. Photobiol*. **2004**, *80*(3), 422–428.

Hemmes, J.H.; Winkler, K.C.; Kool, S.M. Virus Survival as a Seasonal Factor in Influenza and Poliomyelitis. *Nature* **1960**, *188*, 430–431.

Hicke, J.A.; Slusser, J.; Lantz, K.; Pascual, F.G. Trends and Interannual Variability in Surface UVB Radiation Over 8 to 11 Years Observed Across the United States. *J.Geophys. Res.* **2008**, *113,* D21302.

Jagger, J. *Solar-UV Actions on Living Cells.* Praeger: New York, 1985.

King, B.; Kesavan, J.; Sagripanti, J.-L. Germicidal UV Sensitivity of Bacteria in Aerosols and on Contaminated Surfaces. *Aerosol Sci. Technol.* **2011**, *45*, 1–9.

Ko, G.; Melvin, F.W.; Burge, H.A. The Characterization of Upper-Room Ultraviolet Germicidal Irradiation in Inactivating Airborne Microorganisms. *Environ. Health Perspect.* **2002**, *110*(1), 95–101.

Kowalski, W. *Ultraviolet Germicidal Irradiation Handbook: UVGI for Air and Surface Disinfection*. Springer: New York, 2009.

Kratz, R.F. *Barron's E-Z Microbiology*. Barron's Educational Series: New York, 2005.

Lin, C.; Li, C. Control Effectiveness of Ultraviolet Germicidal Irradiation on Bioaerosols. *Aerosol Sci. Technol.* **2002**, *36*(4), 474–478.

Lubin, D; Jensen, E.H. Effects of Clouds and Stratospheric Ozone Depletion on Ultraviolet Radiation Trends. *Nature* **1995**, *377*, 710–713.

Lytle, D.C.; Sagripanti, J.-L. Predicted Inactivation of Viruses of Relevance to Biodefense by Solar Radiation. *J. Virol.* **2005**, *79*(22), 14244–14252.

Masschelein, W.J.; Rice, R. *Ultraviolet Light in Water and Wastewater Sanitation*. CRC Press: Boca Raton, FL, 2002.

McDevitt, J.J.; Lai, K.M.; Rudnick, S.N.; Houseman, E; Andres, M.W.; Milton, D.K. Characterization of UVC Light Sensitivity of Vaccinia Virus. *Appl. Environ. Microbiol.* **2007**, *73*(18), 5760–5766.

McLean, R.L. Comments on Reducing Influenza Epidemics Among Hospitalized Veterans by UV Irradiation of Droplets in the Air. *Amer. Rev. Respir. Dis.* **1956**, *83*(Suppl.), 36–38.

Menetrez, M.Y.; Foarde, K.K.; Webber, T.D.; Dean, T.R.; Betancourt, D.A. Efficiency of UV Irradiation on Eight Species of *Bacillus*. *J. Environ. Eng. Sci.* **2006**, *5*, 329–334.

Menzies, D.; Popa, J.; Hanley, J.A.; Rand, T; Milton, D.K. Effect of Ultraviolet Germicidal Lights Installed in Office Ventilation Systems on Workers' Health and Wellbeing: Double-Blind Multiple Crossover Trial. *Lancet* **2003**, *362*, 1785–1791.

Miller S.L.; Macher, J.M. Evaluation of a Methodology for Quantifying the Effect of Room Air Ultraviolet Germicidal Irradiation on Airborne Bacteria. *Aerosol Sci. Technol.***2000**, *33*, 274–295.

Mims, F.M. Avian Influenza and UV-B Blocked by Biomass Smoke. *Environ. Health Perspect.* **2005**, *113*(12), A806–A807.

Moeller, R.; Horneck, G.; Facius, R.; Stackebrandt, E. Role of Pigmentation in Protecting *Bacillus* sp. Endospores Against Environmental UV Radiation. *FEMS Microbiol. Ecol.* **2005**, *51*, 231–236.

Munakata, N. Comparative Measurements of Solar UV Radiation with Spore Dosimetry at Three European and Two Japanese Sites. *J. Photochem. Photobiol B.* **1999**, *53*, 7–11.

Munakata, N.; Kazadzis, S.; Bais, A.F.; Hieda, K.; Ronto, G.; Rettberg, P.; Horneck, G. Comparisons of Spore Dosimetry and Spectral Photometry of Solar-UV Radiation at Four Sites in Japan and Europe. *Photochem. Photobiol.* **2000**, *72*(6), 739–745.

Munakata, N.; Makita, K.; Bolsee, D.; Gillotay, D.; Horneck, G. Spore Dosimetry of Solar UV Radiation: Applications to Monitoring of Daily Irradiance and Personal Exposure. *Adv. Space Res.* **2000**, *26*(12), 1995–2003.

Nicholson, W.L.; Galeano, B. UV Resistance of *Bacillus anthracis* Spores Revisited: Validation of *Bacillus subtilis* Spores as UV Surrogates for Spores of *B. anthracis* Sterne. *Appl. Environ. Microbiol.* **2003**, *69*(2),1327–1330.

Nicholson, W.L.; Setlow, B.; Setlow, P. UV Photochemistry of DNA in vitro and in *Bacillus subtilis* Spores at Earth Ambient and Low Atmospheric Pressure: Implications for Spore Survival on Other Planets or Moons in the Solar System. *Astrobiology* **2002**, *2*, 417–425.

Peccia, J.; Hernandez, M. Photoreactivation in Airborne *Mycobacterium parafortuitum*. *Appl. Environ. Microbiol.* **2001**, *67*(9), 4225–4232.

Puskeppeleit, M.; Quintern, L.E.; Naggar, S.E.; Schott, J.-U.; Eschweiler, U.; Horneck, G. Long-Term Dosimetry of Solar UV Radiation in Antarctica with Spores of *Bacillus subtilis*. *Appl. Environ. Microbiol.* **1992**, *58*(8), 2355–2359.

Rauth, A.M. The Physical State of Viral Nucleic Acid and the Sensitivity of Viruses to Ultraviolet Light. *Biophys. J.* **1965**, *5*, 257–273.

Remsen, J.F.; Miller, N.; Cerutti, P.A. Photohydration of Uridine in the RNA of Coliphage R17, II. The Relationship Between Ultraviolet Inactivation and Uridine Photohydration. *Proc. Natl. Acad. Sci. USA* **1970**, *65*(2), 460–466.

Riesenman P.J.; Nicholson, W.L. Role of the Spore Coat Layers in *Bacillus subtilis* Spore Resistance of Hydrogen Peroxide, Artificial UV-C, UV-B, and Solar UV Radiation. *Appl. Environ. Microbiol.* **2000**, *66*(2), 620–626.

Rutala, W.A.; Gergen, M.F.; Weber, D.J. Room Decontamination with UV Radiation. *Infect. Control Hosp. Epidemiol.* **2010**, *31*(10), 1025–1029.

Sagripanti, J.-L.; Lytle, C.D. Inactivation of Influenza Virus by Solar Radiation. *Photochem. Photobiol.* **2007**, *83*, 1278–1282.

Schreier, W.J.; Schrader, T.E.; Koller, F.O.; Gilch, P.; Crespo-Hernandez, C.E.; Swaminathan, V.N.; Carell, T.; Zinth, W.; Kohler, B. Thymine Dimerization in DNA is an Ultrafast Photoreaction. *Science* **2007**, *315*(5812), 625–629.

Setlow, P. Spore Germination. *Curr. Opin. Microbiol.* **2003**, *6*, 550–556.

Setlow, P. Spores of *Bacillus subtilis*: Their Resistance to and Killing by Radiation, Heat, and Chemicals. *J. Appl. Microbiol.* **2006**, *101,* 514–525.

Siegal, J.D.; Rhinehart, E.; Jacckson, M.; Chiarello, L., and the Healthcare Infection Control Practices Advisory Committee. Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. 2007, http://www.cdc.gov/ncidod/dhqp/pdf/isolation2007. pdf (accessed June 2012).

TB Infection-Control Guidelines Work Group. Guidelines for Preventing the Transmission of *Mycobacterium* Tuberculosis in Health-Care Facilities. *MMWR Morb. Mortal Wkly. Rep.* **1994**, *43*(RR-13), 1–132.

Tseng, C.; Li, C. Inactivation of Viruses on Surfaces by Ultraviolet Germicidal Irradiation. *J. Occup. Environ. Hyg.* **2007**, *4*, 400–405.

Tucker, J.B.; Zilinskas R.A. The 1971 Smallpox Outbreak in the Soviet City of Aralsk: Implications for Variola Virus as a Bioterrorist Threat. *Crit. Rev. Microbiol.* **2003**, *29*, 81–95.

U.S. Environmental Protection Agency. *Investigation of Simulated Sunlight in the Inactivation of* B. anthracis *and* B. subtilis *on Outdoor Materials.* Report EPA/600/R-10/048. Office of Research and Development, National Homeland Security Research Center: Washington, DC, 2010.

UV-B Monitoring and Research Program website, http://UVB.nrel.colostate.edu (accessed June 2012).

Xu, P.; Kujundzic, E.; Peccia, J.; Schafer, M.P.; Moss, G.; Hernandez, M.; Miller, S.L. Impact of Environmental Factors on Efficacy of Upper-Room Air Ultraviolet Germicidal Irradiation for Inactivation Airborne Mycobacteria. *Environ. Sci. Technol.* **2005**, *39*, 9656–9664.

Xu, P.; Peccia, J.; Fabian, P.; Martyny, J.W.; Fennelly, K.P.; Hernandez, M.; Miller S.L. Efficacy of Ultraviolet Germicidal Irradiation of Upper-Room Air in Inactivating Airborne Bacterial Spores and Mycobacteria in Full-Scale Studies. *Atmos. Environ.* **2003**, *37*, 405–419.

Xue, Y.; Nicholson, W.L. The Two Major Spore DNA Repair Pathways, Nucleotide Excision Repair and Spore Photoproduct Lyase, are Sufficient for the Resistance of *Bacillus subtilis* Spores to Artificial UV-C and UV-B But not to Solar Radiation. *Appl. Environ. Microbiol.* **1996**, *62*(7), 2221–2227.